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Effects of Exogenous Enzymatic Treatment During Processing on the Sensory Quality of Summer Tieguanyin Oolong Tea from the Chinese Anxi County

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Summary

In order to attenuate the bitter taste and improve the aroma of the summer tieguanyin oolong tea from the Chinese Anxi county, the effects of processing treatment with exogenous laccase and α-galactosidase on tea sensory quality and related compounds were investigated. The solutions of laccase and/or α-galactosidase were sprayed on the tea leaves before the first drying process. The sensory evaluation results showed that the sensory quality of the tea was significantly enhanced with the enzymatic treatment. The combined application of laccase at 8.25 and α-galactosidase at 22 U per kg of fresh tea shoots achieved the most satisfying sensory quality. Further analysis of flavour-related constituents was carried out by HPLC and GC-MS. The HPLC analysis showed that the contents of catechins and total polyphenols were reduced, compared to the untreated group, by 11.9 and 13.3 % respectively, and the total soluble sugars and water extract content were increased by 19.4 and 6.6 % respectively, after the treatment with both enzymes. The decrease of catechins and total polyphenols reduced the bitterness and astringency of the summer tea, while the increase of total soluble sugars and water extract content improved the sweetness and mellow taste. The aromatic compound data from GC-MS showed that the total essential oil content in these tea samples co-treated with laccase and α-galactosidase increased significantly, in which aldehydes, alcohols, esters and alkenes increased by 23.28, 37.05, 20.10 and 38.99 %, respectively. Our data suggest that the exogenous enzymatic treatment can enhance the summer oolong tea quality, especially its taste and aroma.

Key words: summer tieguanyin oolong tea, exogenous enzymatic treatment, laccase, α-galactosidase, sensory quality

Introduction

Tea, a popular healthy beverage consumed around the world, is classified into six types: green tea, yellow tea, dark tea (including brick tea and pu-erh tea), white tea, oolong tea and black tea based on the degree of fermentation (oxidation) during processing (*1*). These types of tea have different quality characteristics including colour, aroma, taste and appearance. Many studies have shown the positive effects of green and black tea on oxidative and inflammatory stress, insulin resistance and lipid metabolism (*2*). Oolong tea has proven its effects on antioxidation, lipid reduction and anti-obesity (*3*).

The fresh tea leaves are rich in catechins (flavan-3--ols), caffeine and theanine (*N*-ethyl-γ-L-glutamine), which contribute to the main health-promoting functions and sensory quality of tea. Moreover, there are abundant gly-

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cosides with monoterpene alcohols and aryl alcohols like aglycone, which having been hydrolysed by endogenous glucosidase release the aglycone during processing. These compounds have floral or fruity smell and dominate the formation of tea aroma (*4*). During the processing period of oolong tea, fresh tea leaves are harvested and undergo the basic steps of being withered, bruised (shaken), partially fermented, fixed, rolled and dried. Oxidation is limited only to the leaf edges through shaking, known as 'semi-fermentation'. Tieguanyin tea, a kind of oolong tea that originates from Anxi county (Fujian province, PR China), is one of the top ten famous teas in China and is processed from Tieguanyin cultivar. Besides natural conditions and good agricultural practices of tea plantations, the season of tea production plays an important role in quality. According to the harvest time, tieguanyin tea is classified into spring, summer and autumn tea. It has been found that summer tea has higher output than spring and autumn tea, but generally has lower quality (*5*), the difference being that summer tea has fewer soluble sugars and more polyphenols and caffeine, undesirable bitter and astringent taste with lower quality aroma. Given the situation, it is necessary and justifiable to find ways to improve the sensory quality of summer tieguanyin tea.

To optimize the unique quality of tea, endogenous enzymes like polyphenol oxidase and glucosidase in tea shoots should be well controlled. However, this is restricted by the quality of fresh leaves and the production process. Many attempts such as changing the processing conditions have been made to improve the quality of summer tea (*6,7*). In addition, it has been reported that the quality of tea and related tea products has been improved by introducing some exogenous enzymes such as polyphenol oxidase, peroxidase, tannase, cellulase, pectinase, protease and β-glucosidase into tea processing since 1990s. For example, Murugesan *et al.* (*8*) did screening experiments with a series of exogenous enzymes and found that the purified cellulase combined with laccase from *Trametes versicolor* in the ratio of 3:2 (by volume) was the most effective in enhancing the quality of black tea. In 2013, Kumar *et al.* (*9*) reviewed various major enzymatic methods applied in the ready-to-drink black tea production and discussed the advantages and limitations of these methods. As far as green tea is concerned, β-glucosidase could significantly enhance the aromatic constituents in green tea liquor (*10*), papain and cellulase could reduce its bitterness (*11*), and the proteolytic enzyme derived from *Aspergillus oryzae* could hydrolyse proteins in green tea residues (*12*). Besides, peroxidase, cellulase and Flavourzyme® were employed by Li and Liu in 2008 (*13*) in the processing of pu-erh tea to improve its quality. Above all, exogenous enzyme treatment can achieve the purpose of improving tea quality by changing the contents of some constituents in the tea.

Laccase is one of the copper-containing polyphenol oxidases (*p*-diphenol oxidase, EC 1.10.3.2), which was first found in sumac juice in 1883 (*14*) and is widely present in fungi and bacteria (*15,16*). Its substrates include, among others, polyphenols, methoxy-substituted phenols and diamines. Laccase is able to transform phenols by self-coupling or cross-coupling with polymeric products. Canfora *et al.* (*17*) proved that laccase was effective in the transformation of simple and complex phenolic mixtures. Luo *et al.* (*18*) studied the effect of the kinetics of biocatalytic activity of laccase during oxidation of phenolic compounds and demonstrated that laccase-catalysed oxidation of phenolic compounds was more effective than that of epicatechin and catechol. Yue (*19*) performed the study on synthesising theaflavins by using laccase. Therefore, laccase has been widely used in food industry (*20–22*), such as for juice clarification, beer processing stability and food sensory quality improvement. It can deoxygenate oil, remove bitterness and other unpleasant tastes of cacao, reduce the bitterness of olives, improve the colour and enhance the quality of black tea (*8*).

The α-galactosidase (EC 3.2.1.22), originally from the yeast fermentation liquid, exists in many animals, plants and microorganisms. It can specifically hydrolyse α-galactosidase bond and has been widely applied in food, feed and medical industry (*23–25*). In food processing, α-galactosidase is used mostly to remove 'flatus-producing factor' in soya bean, thereby reducing soya bean oligosaccharides and making it easily digestible (*26–28*).

To the best of our knowledge, so far there have been no reports on the effects of processing treatment of exogenous laccase and α-galactosidase on the sensory quality of summer tieguanyin tea from the Anxi county in China. In order to improve the fragrance quality of the tea by reducing bitterness and astringency, we investigated the effects of laccase and α-galactosidase on the final tea quality. The main constituents related to its taste and aroma were also analysed.

Materials and Methods

Materials

Summer fresh tea shoots (*Camellia sinensis* cv. Tieguanyin), consisting of dormant bud and three or four adjacent leaves, were hand-plucked in July 2011 from the fields of the Xianghua Tea Factory, Anxi Tie Guan Yin Group, Anxi, Fujian province, PR China.

Laccase 5000 U/g and α-galactosidase 5000 U/g were purchased from Aidun Biology Engineering Company (Jiangsu, PR China). Glucose, catechin (including epigallocatechin, epicatechin, epigallocatechin gallate and epicatechin gallate), caffeine, theanine and gallic acid standards were purchased from Sigma-Aldrich (Shanghai, PR China). Other reagents were of HPLC or analytical grade. Ultrapure Milli-Q Direct 8 water was produced by Merck Millipore (Billerica, MA, USA).

Enzymatic treatment

The basic manufacturing process for summer fresh tea shoots according to Chinese National Standard GB/T 19598-2006 (*29*) includes the following steps: plucking→withering and cooling→bruising and rolling→fixation→twisting and wrapping→first drying→full drying. For enzymatic treatment, 10 kg of half-dried tea were sprayed with 220 mL of aqueous solution containing enzymes prior to full drying, rolled for 2 min and left for 10 min in tea baking box, and then dried fully at 70–80 °C.

As shown in Table 1, the sample not treated with the enzymes was labelled CK, the groups treated with laccase (0.05, 0.1 and 0.2 g/L) A1, A2 and A3 respectively, the groups treated with α-galactosidase (0.1, 0.2 and 0.4 g/L) B1, B2 and B3, and the groups treated with both laccase and α-galactosidase (in g/L: laccase 0.075+α-galactosidase 0.1 and laccase 0.075+α-galactosidase 0.2) C and D respectively. Three parallel experiments were done at each concentration.

Table 1. Concentrations of enzymes used for the treatment of different summer tieguanyin tea samples

Sample	γ(enzyme)/(g/L)
CK	Not treated enzymatically
A1	Laccase 0.05
A2	Laccase 0.1
A3	Laccase 0.2
B1	α-galactosidase 0.1
B2	α-galactosidase 0.2
B3	α-galactosidase 0.4
C	Laccase 0.075+α-galactosidase 0.1
D	Laccase 0.075+α-galactosidase 0.2

Sensory evaluation

The tea samples were examined and scored independently according to Chinese National Standard GB/T 23776-2009 (*30*) by a tea tasting panel consisting of six experts from the Department of Tea Science, Anhui Agricultural University, Hefei, PR China. The tea tasters firstly evaluated the appearance of the dry leaves, including colour, size and twist. A mass of 5.0 g of tea samples was infused three times (for 2, 3 and 5 min) in a 110-mL tea tasting porcelain cup with 110 mL of freshly boiled water, and then the infusions were poured three more times into 110-mL tea tasting porcelain bowls for quality assessment. Finally, the tea tasters assessed the aroma, colour and taste of the infusion based predominantly on the infusion time of 3 min. The grading system was based on the maximum score of 100 for each quality attribute (aroma and taste).

Determination of total polyphenolic content

The total polyphenols, caffeine, catechins, free amino acids, tea polysaccharides and water extracts of the tea samples were examined by National Tea Quality Supervision and Inspection Center, Fujian, PR China. The total polyphenolic content (TPC) was determined by spectrophotometry (722S, Shanghai Analytical Instrument Company, Shanghai, PR China), using gallic acid as the standard, according to the method described in the International Standard ISO 14502-1:2005 (*31*). Briefly, 1.0 mL of the diluted samples was transferred in duplicate to separate tubes containing 5.0 mL of a 1:10 dilution of Folin–Ciocalteu reagent in water. Then, 4.0 mL of a sodium carbonate solution (7.5 %, by mass per volume) were added. The tubes were then allowed to stand at room temperature for 60 min before absorbance was measured at 765 nm against water. The TPC was expressed as gallic acid equivalents (GAE) in g per 100 g of material. The concentration of polyphenols in the samples was derived from a standard curve of gallic acid ranging from 10 to 60 μg/mL (Pearson's correlation coefficient: R^2=0.998).

Determination of caffeine and catechins

Caffeine and catechins were quantified by Chinese National Standard GB/T 8312-2013 (*32*) and International Standard ISO 14502-2:2005, respectively (*33*). Caffeine and catechins were analysed by a HPLC system (2695/2996, PDA detector, Waters, Milford, MA, USA) equipped with a reversed phase Waters C_{18} column (5 μm, 250 mm× 4.6 mm i.d.) at 35 °C and 278 nm. Concentrations of caffeine and catechins were quantified by their peak areas against those of standards prepared from authentic compounds.

Determination of water extract, tea polysaccharides and free amino acids

Water extract was determined according to Chinese National Standard GB/T 8305-2013 (*34*). Briefly, a volume of 50 mL of tea extract prepared as described above was evaporated in a dish on boiling water bath to partially dry, and further dried in an oven at 103 °C to complete dryness and then weighed after cooling down to room temperature in a silica gel desiccator.

Tea polysaccharides were determined by an anthrone-sulphuric acid method described by Wang *et al*. (*35*). A volume of 4 mL of 0.033 % anthrone in concentrated sulphuric acid was added to 1 mL of tea polysaccharide solution and heated for 7 min at 100 °C. The absorbance was measured at 620 nm after cooling for 30 min, and glucose was used as the standard. The result was expressed in g of glucose per 100 g of material and derived from a standard curve of glucose ranging from 0.025 to 0.2 mg/mL (Pearson's correlation coefficient: R^2=0.999).

The content of free amino acids was determined spectrophotometrically at 570 nm with ninhydrin colourimetry, using theanine as standard, according to Chinese National Standard GB/T 8314-2013 (*36*). It was expressed in g of theanine per 100 g of material and derived from a standard curve of theanine ranging from 0.004 to 0.012 mg/mL (Pearson's correlation coefficient: R^2=0.985).

Determination of aromatic compounds

The aromatic compounds were analysed and determined by brewed extraction method reported by Kawakami *et al.* (*37*). In brief, a simultaneous steam distillation and extraction apparatus was used to extract volatile compounds with ethyl ether as the extraction solvent, and then the extracts were concentrated. Finally, the concentrates were determined by gas chromatography-mass spectrometry (GC-MS, QP2010S, Shimadzu, Kyoto, Japan).

Aromatic compounds were extracted as follows: 30 g of tea sample were mixed with 500 mL of deionized water in a 1-litre flask, and 1 mL of ethyl decanoate solution (50 μg/mL in ethyl ether) was added as an internal standard for GC-MS quantitative analysis. Then, the volatile constituents were extracted applying simultaneous distillation and extraction method using a modified Likens-

-Nickerson apparatus. A volume of 30 mL of redistilled ethyl ether was used as the extraction solvent, and the extraction was continued for 1 h after the sample began boiling in the flask. The ethyl ether phase was then transferred into a 50-mL glass tube and dehydrated with 5 g of anhydrous sodium sulphate overnight. The dehydrated ethyl ether phase was then concentrated to 1 mL. The concentrate was used for GC-MS analysis.

The flavour substances were analysed by GC-MS, with DB-5 chromatographic column (30 m×0.25 mm×0.25 μm i.d.). The oven temperature was kept at 50 °C for 2 min, then raised to 60 °C at a rate of 1 °C per min, maintained for 2 min, then raised to 150 °C at a rate of 3 °C per min, and maintained for 5 min, then raised to 250 °C at a rate of 10 °C per min and maintained for 7 min. The injector temperature was 250 °C. The temperature of the detector was 280 °C. Highly purified helium was used as carrier gas with a constant flow rate at 1.0 mL/min. The split ratio was 1:10 and the injection volume was 1 μL. Mass spectrometry was performed in electron ionization mode by scanning the mass spectra at 70 eV in an *m*/*z* range from 35 to 400. The aroma constituents were identified by matching GC and MS data with those of authentic compounds and the literature. The relative amount of each compound was calculated by the ratio of the compound peak area to the internal standard (ethyl decanoate) peak area.

Data analysis

The experimental data about taste were shown as the average value±standard deviation (*N*=3). Data were analysed using data processing system (DPS) for analysis of variance (ANOVA), with statistically significant difference at $p<0.05$.

The aromatic constituents were identified by NIST l47 and NIST 27 libraries (*38*).

Results and Discussion

Tea taste and aroma are the most important indices to evaluate the sensory quality of oolong tea. Taste reflects the coordination effects of various water-soluble substances in tea infusion, which include soluble phenols, caffeine, amino acids, soluble sugars, *etc*. Sensory aroma characteristics of tea depend on the total amount of essential oil and the abundance of various aromatic compounds.

Summer tieguanyin tea has plain and thin but bitter and astringent taste and plain aroma. In order to improve the quality of summer tea, the exogenous enzymes laccase and α-galactosidase were added to adjust the content of one or several taste and aromatic factors.

Sensory evaluation results of tea samples

The sensory evaluation results of the samples CK, A1, A2, A3, B1, B2, B3, C and D are presented in Table 2 (the sensory tests were conducted according to Chinese National Standard GB/T 14487-2008 (*39*)) and they indicate that enzymatic treatments had significant effects on the sensory quality.

The taste of sample CK was described as bitter and astringent with significant yin flavour, and its score was 83. In contrast, astringency decreased with the increase of laccase concentration, while bitterness decreased with the increase of α-galactosidase concentration. The taste scores of A1, A2, A3, B1, B2 and B3 were 84, 86, 87, 87, 89 and 82, respectively; sample C was mellow and slightly bitter, with score of 91; sample D was mellow, sweet and with significant yin flavour, with a taste score of 93.

The aroma of sample CK was described as clean and pure and its score was 85; the aroma became stronger with the increase of α-galactosidase concentration, but sample B3 was described as light; the aroma scores of A1, A2, A3, B1, B2 and B3 were 86, 88, 87, 89, 92 and 89, respectively; both samples C and D had intense and lasting aroma, and their scores were 91 and 93, respectively. In summary, these results indicate that the sensory quality of sample D was the best. Both laccase and α-galactosidase showed significant effects on improving the sensory quality of Anxi summer tieguanyin tea. Laccase had a positive effect on the taste only, while α-galactosidase had a positive effect mainly on the aroma and only partly affected the taste. Moreover, the experiments showed that the concentration of enzymatic solution was an important factor to influence the effects of exogenous enzymatic treatment on the sensory quality. When the mixed enzyme solution with 0.075 g/L of laccase and 0.2 g/L of α-galactosidase was used, the tea received the highest score.

Table 2. Results of sensory evaluation of the summer tieguanyin tea from Anxi county after different enzymatic treatments

Sample	Flavour		Aroma	
	Sensory evaluation terms	Score	Sensory evaluation terms	Score
CK	plain and thin, bitter and astringent, significant yin flavour	83	clean and pure	85
A1	not mellow enough, shows bitterness, slightly astringent, some yin flavour	84	pure and normal	86
A2	not mellow enough, shows bitterness, some yin flavour	86	clean aroma	88
A3	mellow and thick, slightly bitter, significant yin flavour	87	clean aroma, less pure	87
B1	not mellow and thick enough, slightly astringent, some yin flavour	87	clean aroma, not lasting enough	89
B2	mellow and thick, slightly astringent, significant yin flavour	89	intense aroma	92
B3	mellow, plain and thin, slightly sweet, some yin flavour	82	light	89
C	heavy and mellow, slightly bitter, some yin flavour	91	intense and lasting aroma	91
D	sweet, mellow and fresh, significant yin flavour	93	intense and lasting aroma	93

For sample abbreviations see legend of Fig. 1

Effects of enzymatic treatment on the taste of tieguanyin tea

As shown in Fig. 1, the contents of tea polyphenols in samples A1, A2 and A3, which were treated with different concentrations of laccase, were 12.3, 11.9 and 11.5 %, lower than the sample CK (13.4 %), their catechin content was 7.93, 7.29 and 6.73 % respectively, which was lower than that of the sample CK (8.32 %), while the contents of tea polyphenols and catechin significantly decreased along with the increase of laccase concentration. Therefore, laccase treatment can reduce the content of tea polyphenols and this effect is concentration-dependent.

Similar results can also be observed in the samples treated with α-galactosidase, marked B1, B2 and B3. The content of tea polyphenols in sample CK was 13.4 % and it slightly decreased to 12.8, 12.8 and 12.7 % after a treatment with different concentrations of α-galactosidase (Fig. 1). However, the catechin content in samples B1, B2 and B3 (8.24, 8.23 and 8.16 %, respectively) was slightly lower compared with sample CK (8.32 %), but this was not statistically significant. These results demonstrated that the effects of α-galactosidase treatment on the content of tea polyphenols and catechins were relatively weak.

When the combination of these two enzymes (samples C and D) was used, the content of both tea polyphenols and catechins decreased significantly compared with sample CK. When the concentration of α-galactosidase (sample C) was 0.1 g/L, the reduction rate was similar to that of the samples treated with laccase only. However, after 0.2 g/L of α-galactosidase (sample D) was sprayed together with laccase, lower content of tea polyphenols and catechins was determined, which indicated that although the effect of α-galactosidase alone on the summer tea was weak, it was able to enhance the effects of laccase on the content of tea polyphenols and catechins when these two enzymes were applied together.

As shown in Fig. 1, the total soluble sugars of samples treated with laccase alone (samples A1, A2 and A3), α-galactosidase alone (samples B1, B2 and B3) or the combination of the two enzymes (samples C and D) were significantly higher than in the CK sample (6.5, 6.9 and 7.6 %

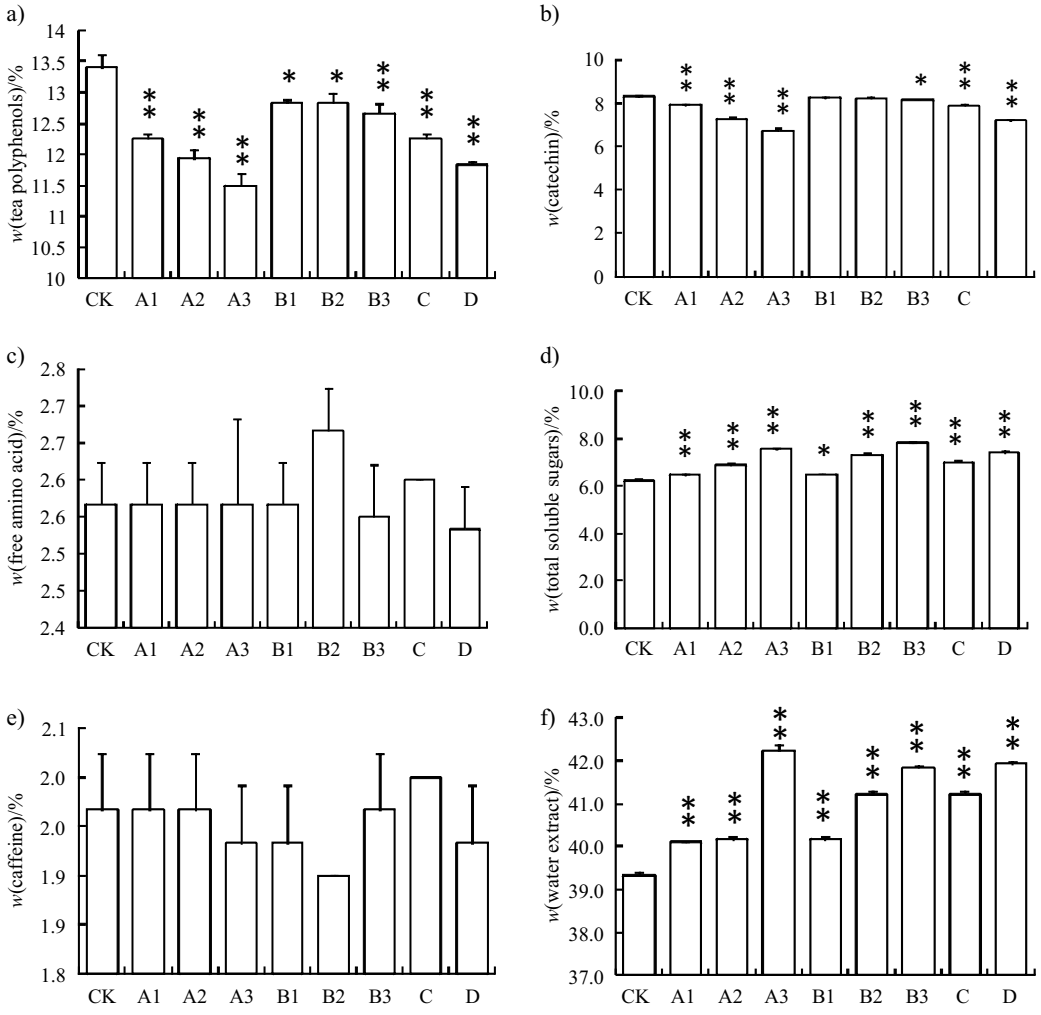

Fig. 1. Components in summer tieguanyin oolong tea treated with different enzyme concentrations (in g/L): not treated (CK), treated with laccase 0.05 (A1), treated with laccase 0.1 (A2), treated with laccase 0.2 (A3), treated with α-galactosidase 0.1 (B1), treated with α-galactosidase 0.2 (B2), treated with α-galactosidase 0.4 (B3), treated with laccase 0.075 and α-galactosidase 0.1 (C), treated with laccase 0.075 and α-galactosidase 0.2 (D).
*significant difference (*t*-test, $p<0.05$), **very significant difference (*t*-test, $p<0.01$)

in samples A1–A3, 6.5, 7.3 and 7.8 % in samples B1–B3, 7.0 and 7.4 % in samples C and D, respectively, *vs.* 6.2 % in sample CK). The same enhancement effects could be obtained for water extract content (40.1, 40.2 and 42.2 % in samples A1–A3, 40.2, 41.2 and 41.8 % in samples B1–B3, 41.2 and 41.9 % in samples C and D, respectively, *vs.* 39.3 % in sample CK). The total soluble sugars and the content of water extract increased by 19.4 and 6.6 % after the treatment with combined enzymes. The increased level of water extract in samples treated with laccase was higher than in the samples treated with α-galactosidase, but the increased level of total soluble sugars in samples treated with laccase was lower than in samples treated with α-galactosidase. Unlike these two results above, the content of caffeine and free amino acids was basically the same whether they were treated with laccase or α-galactosidase.

It can be seen from the above results that the content of soluble polyphenols decreased after laccase treatment. After enzymatic treatment, the total soluble sugars and the content of water extract increased significantly, while the change of caffeine and free amino acids was not obvious. Decrease of catechins and polyphenols in tea could effectively reduce the bitterness and astringency of the summer tea; increase of the total soluble sugars and the content of the water extract could balance the flavour of tea infusion. The reason why α-galactosidase treatment could result in an increase of total soluble sugars may be that α-galactosidase can hydrolyse α-glycosidic bond specifically, as has been previously reported in the literature. Both laccase and α-galactosidase can destruct the cell wall structure and promote the release of intracellular nutrients, so the content of the water extract was significantly increased after enzymatic treatment.

Low quality of summer tea is difficult to improve only by adjusting the processing parameters; however, it has been reported that a treatment with exogenous enzymes can achieve the purpose by changing the contents of some constituents in the tea. The study found that the reason for the bitterness and astringency of Anxi summer tieguanyin tea is that the summer tea has lower soluble sugars and higher polyphenol content (*40*). Research has also shown that there are positive effects of the higher total soluble sugar ratio of tea polyphenols. The above results show that appropriate exogenous laccase and α-galactosidase treatment could achieve the expected result of adjusting the content of polyphenols and total soluble sugars, which was proven by the sensory evaluation results.

Effects of enzymatic treatment on the aroma of tieguanyin tea

Fresh tea leaves are virtually odourless and the aroma components of tea are generated mainly because of biochemical and chemical reactions during tea processing. In our experiments, after the addition of exogenous enzymes, the aroma precursors were transformed into aroma components. Sixty-seven compounds were identified which can be divided into eight groups according to their structure: 6 aldehydes, 16 alcohols, 20 esters, 7 ketones, 1 acid, 5 alkenes, 3 alkanes and 9 other compounds. Almost all the compounds have been identified as the aroma compounds of tea by Hara *et al.* (*41*). The main volatile constituents of the samples are shown in Table 3 and Fig. 2. The results illustrate that the relative content (values given as the ratio of the component peak area to the internal standard (ethyl decanoate) peak area) of the total essential oils in samples A1 (108.19), A2 (113.41), A3 (117.65), B1 (111.00), B2 (129.83), B3 (135.84), C (117.15) and D (123.26) was higher than that in sample CK (97.95). Compared to those in the control group, the total essential oils of tea sample D increased significantly, among which aldehydes increased by 23.28 %, alcohols by 37.05 %, esters by 20.10 % and alkenes by 38.99 %.

The main constituents of the essential oils were aldehydes, alcohols, esters and alkenes, which accounted for 70–80 %. Among them, the contents of aldehydes, alcohols, esters and alkenes in the samples treated with laccase and α-galactosidase were obviously higher than in the CK sample. The samples treated with α-galactosidase had a higher content of aldehydes and alcohols, but lower content of esters than those treated with laccase.

Nerolidol, indole and α-farnesene were found to be the most important odour-active compounds in oolong tea (*37*). The smell of nerolidol is like the light fragrance of roses and apples, which is very sweet, refreshing and lasting. Indole can enhance the flavour of tea. The organoleptic character of farnesene is soft and mellow. Compared with sample CK, all these three compounds were increased in sample D (53.34 *vs.* 38.09 % of nerolidol, 11.52 *vs.* 10.33 % of indole, and 12.90 *vs.* 9.21 % of farnesene). Benzonitrile is considered as the unique constituent of tieguanyin (*42*), which was also detected in this study. Therefore, these odourants were the most important components of tieguanyin tea. The contents of these components in the samples treated with α-galactosidase were much higher than in those treated with laccase and the sample CK. It is obvious that α-galactosidase plays an important role in tea aroma formation.

Alcohols accounted for about half of the total essential oils. Linalool, phenylethyl alcohol, nerolidol and phytol were the main constituents of oolong tea. It is known that terpene alcohols, such as linalool can be generated by the hydrolysis of their glycosides, which are precursors of tea aroma, through the enzymatic action (*43*). Fig. 2 shows that the linalool, phenylethyl alcohol and nerolidol content greatly increased in the samples B2 and B3. Phenylethyl alcohol was considered as a tea aroma precursor (*44*), which increased in samples treated with enzymes. Nerolidol is the major component of volatile oil contributing to the odour of tieguanyin and is the most important indicator of the aroma of Fujian oolong tea (*45*). The content of phytol, the hydrolysed product of chlorophyll, was 8.51 % in sample A3, which was much higher than that in sample CK (6.01 %). In the above sensory evaluation, the colour of sample D was golden yellow, brighter than that of sample CK, and this change resulted from the effect of laccase.

Aldehydes accounted for above 10 % of the total essential oils. (E)-2-hexenal, nonanal, palmitaldehyde and diallyl acetal belong to aliphatic aldehydes. (E)-2-hexenal is known to possess a strong green note. The relative level of (E)-2-hexenal is much higher in sample D (0.39) than in sample CK (0.25). Nonanal gives a pleasant rose note.

Table 3. Aroma compounds and relative contents of tieguanyin tea samples from Anxi county after different enzymatic treatments

No.	RT/min	Compound	Relative content*								
			CK	A1	A2	A3	B1	B2	B3	C	D
1	5.691	(E)-2-hexenal	0.25	0.26	0.26	0.27	0.27	0.17	0.17	0.31	0.23
2	5.825	3-hexen-1-ol	0.09	–	–	–	–	0.08	0.10	0.11	0.06
3	5.852	butyl aldoxime, 3-methyl-, syn-	0.24	0.30	0.34	0.33	0.29	0.14	0.15	0.30	0.15
4	5.925	butyl aldoxime, 2-methyl-, syn-	0.16	0.15	0.14	0.14	0.16	–	–	0.21	–
5	6.545	butyl aldoxime, 3-methyl-, anti-	0.11	0.12	0.13	0.15	0.14	–	0.11	0.16	0.11
6	8.097	isopropyl acetate	0.25	–	–	–	–	0.33	0.35	0.27	0.24
7	10.844	benzaldehyde	0.42	0.31	0.32	0.35	0.32	0.48	0.56	0.49	0.49
8	17.217	benzeneacetaldehyde	9.45	10.12	10.52	11.31	10.56	13.92	15.31	11.46	11.80
9	17.633	(Z)-3,7-dimethyl-1,3,6-octatriene	0.72	0.81	0.91	1.04	0.89	1.31	1.45	0.92	1.24
10	21.223	linalool	0.53	0.46	0.52	0.49	0.57	0.92	0.98	0.61	0.73
11	21.512	nonanal	0.19	0.21	0.21	0.22	0.22	0.35	0.41	0.24	0.33
12	21.996	phenylethyl alcohol	2.36	2.98	3.09	3.16	3.26	3.34	3.58	3.42	2.41
13	22.284	neryl nitrile	0.87	0.90	0.93	1.02	0.90	1.52	1.64	0.98	1.13
14	22.985	glycerin	0.22	–	–	–	–	–	–	–	–
15	23.599	benzyl nitrile	1.06	1.19	1.24	1.34	1.33	1.30	1.36	1.50	1.32
16	25.693	4-*tert*-butyl-1-cyclohexene	0.85	0.89	1.13	1.12	1.21	1.24	1.26	0.99	1.01
17	29.842	isopentyl hexanoate	–	–	–	–	0.02	–	–	–	–
18	30.086	3-phenylpropanol	–	0.32	0.45	–	0.46	–	0.48	–	–
19	30.838	cholestan-22(26)-epoxy-3,16-dione	0.09	–	–	–	–	–	–	–	–
20	31.124	benzyl isocyanide	–	0.21	0.24	0.24	0.29	–	–	–	–
21	32.093	indole	10.33	11.36	11.95	12.45	12.36	14.78	15.01	12.63	11.52
22	32.249	2-phenylnitroethane	1.57	1.62	1.75	1.83	1.83	1.78	1.80	2.04	1.41
23	36.088	(Z)-3-hexenyl hexanoate	0.93	1.42	1.43	1.35	1.40	0.89	0.72	1.50	0.83
24	36.310	hexanoic acid, hexyl ester	0.45	0.85	0.93	0.87	0.92	0.43	0.39	0.98	0.48
25	36.470	(E)-hexanoic acid, 2-hexenyl ester	0.18	0.32	0.31	0.29	0.31	0.15	0.14	0.33	0.17
26	36.671	phenethyl isobutyrate	0.35	0.44	0.43	0.44	0.45	0.22	0.21	0.54	0.24
27	36.850	*cis*-jasmone	0.16	0.15	0.16	0.15	0.16	0.31	0.35	0.19	0.20
28	38.663	β-phenylethyl butyrate	0.44	0.56	0.61	0.58	0.60	0.38	0.33	0.67	0.41
29	40.613	phenethyl-2-methyl butyrate	1.81	2.13	2.19	2.19	2.13	1.55	1.44	2.35	1.69
30	40.874	δ-nonalactone	1.45	1.52	1.64	1.78	1.74	3.93	4.16	1.78	2.25
31	40.958	farnesol	0.56	0.32	0.33	0.39	0.29	–	0.30	0.63	0.63
32	41.527	farnesene	9.21	11.88	12.13	12.76	11.27	9.92	9.28	10.80	12.90
33	42.179	2-allyl-1,3-cyclohexanedione	0.15	0.14	0.15	0.15	0.13	0.16	0.16	0.14	0.14
34	43.550	*p*-menth-4-en-3-one	–	–	–	–	–	0.14	–	–	–
35	43.838	nerolidol	38.09	40.32	41.56	43.23	40.14	54.84	58.92	43.52	53.34
36	44.024	3-hexen-1-ol benzoate	0.21	0.26	0.24	0.24	0.23	0.22	0.19	0.27	0.24
37	44.163	7,8-dihydrolinalool	–	–	–	–	–	0.14	0.10	–	0.09
38	44.293	6,11-dimethyl-2,6,10-dodecatrien-1-ol	0.21	0.32	0.34	0.34	0.32	0.23	0.22	0.31	0.34
39	47.152	2-phenylethyl hexanoate	1.02	1.25	1.31	1.40	1.27	0.92	0.88	1.37	0.96
40	47.510	methyl jasmonate	–	0.21	0.19	–	0.21	0.41	0.46	–	–
41	53.803	neophytadiene	0.26	0.23	0.22	0.35	0.23	0.21	0.19	0.29	0.23
42	53.925	hexahydrofarnesyl acetone	0.19	0.18	0.18	0.20	0.18	0.15	0.13	0.21	0.14
43	53.992	octanoic acid, 2-phenylethyl ester	0.06	0.06	0.06	0.07	0.07	–	–	0.07	–
44	54.162	hydroxyethyl palmitamide	0.25	0.26	0.27	0.31	0.27	0.16	0.16	0.30	0.17
45	54.379	isobutyl phthalate	0.07	0.09	0.13	0.08	0.14	0.10	0.09	0.08	0.05
46	54.562	*trans*-phytol	0.10	0.05	0.07	0.12	0.08	0.10	0.13	0.13	0.10
47	54.954	methyl palmitoleate	–	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.06
48	55.118	2-pentadecanone, 6,10,14-trimethyl-	0.10	–	–	–	–	0.18	0.20	0.14	0.15

Table 3. – continued

No.	RT/min	Compound	Relative content*								
			CK	A1	A2	A3	B1	B2	B3	C	D
49	55.175	farnesylacetone	0.09	0.04	0.04	0.05	–	0.12	0.11	0.11	0.12
50	55.281	methyl palmitate	0.13	0.16	0.18	0.17	0.17	0.19	0.20	0.20	0.25
51	55.596	isophytol	0.31	0.25	0.19	0.28	0.20	0.29	0.36	0.36	0.30
52	55.844	palmitic acid	0.52	0.46	0.41	0.33	0.33	1.20	1.30	0.58	0.71
53	56.709	geranyl linalool isomer B	0.11	0.12	0.16	0.15	0.13	0.15	0.15	0.13	0.21
54	57.408	phytol isomer	0.06	0.04	0.04	0.06	0.04	–	–	–	0.05
55	57.458	methyl linoleate	0.84	1.08	1.36	1.47	1.26	0.88	0.78	1.24	1.34
56	57.541	methyl linolenate	1.83	2.36	2.89	3.19	2.75	2.22	2.03	2.74	2.98
57	57.625	*trans*-piperitol	0.08	–	–	–	–	0.03	0.03	0.05	–
58	57.681	phytol	6.01	6.98	7.57	8.51	6.94	4.56	4.13	6.05	4.92
59	57.999	palmitaldehyde, diallyl acetal	0.48	0.10	0.10	0.12	0.10	0.35	0.46	0.55	0.46
60	58.181	ethyl linoleate	0.08	0.09	0.09	0.09	0.09	–	–	0.10	–
61	58.259	ethyl linoleolate	0.10	0.20	0.22	0.21	0.20	0.10	0.09	0.22	0.06
62	58.417	dodecanamide	–	–	–	–	–	0.20	–	–	–
63	58.649	octadecane	0.09	–	–	–	–	0.17	0.20	0.17	0.16
64	58.777	*cis*-9-hexadecenal	0.12	0.06	0.06	0.06	0.08	0.23	0.28	0.14	0.14
65	59.458	squalene	0.81	0.82	0.82	0.81	0.83	1.17	1.23	0.94	1.09
66	59.583	hexahydronerolidol	0.06	–	–	–	–	0.09	0.09	0.06	0.07
67	61.908	heneicosane	0.22	0.21	0.22	0.16	0.21	0.27	0.28	0.22	0.25
		Total essential oils	97.95	108.19	113.41	117.65	111.00	129.83	135.84	117.15	123.26
		Total aroma compounds	59	55	55	52	55	56	57	57	55

*Values are given as the ratio of the component peak area to the internal standard (ethyl decanoate) peak area; RT=retention time of each peak; –=not detected
For sample abbreviations see legend of Fig. 1

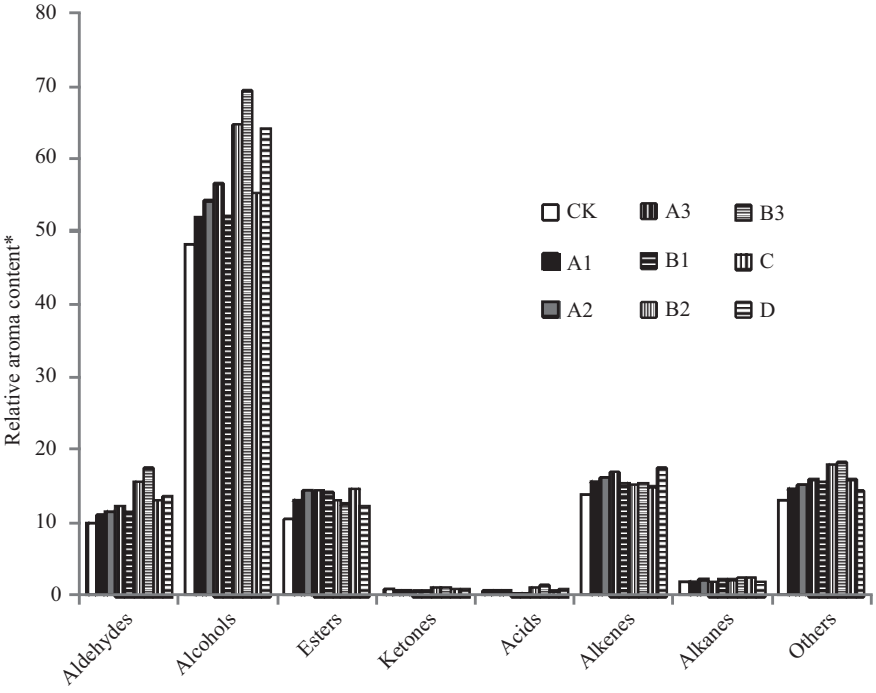

Fig. 2. Comparison of aroma profiles of samples of tieguanyin tea from Anxi county treated with different enzyme concentrations (for sample abbreviations see legend of Fig. 1)
*Values are given as the ratio of the component peak area to the internal standard (ethyl decanoate) peak area

Benzeneacetaldehyde has a characteristic hyacinth-like odour. The contents of nonanal, benzaldehyde and benzeneacetaldehyde in sample D were much higher than in sample CK.

Esters, the main constituents of the essential oils, always have a characteristic pleasant flowery aroma. There were 20 kinds of esters detected in the tea samples. In green tea, oolong tea and black tea, (Z)-3-hexenyl hexanoate has been reported as one of the most important aroma compounds exhibiting a green note, although its concentration was not fairly high (*46–48*). The relative level of (Z)-3-hexenyl hexanoate was much higher in the samples treated with laccase than in sample CK. The contents of methyl palmitate, methyl linoleate and methyl linolenate in samples treated with α-galactosidase and laccase were much higher than in sample CK. These components showed lasting aroma (*49*). Farnesene is the most abundant alkene in tieguanyin oolong tea. It largely increased in samples treated with α-galactosidase or laccase. However, the exception was observed in sample D, in which the content of alkanes decreased.

Research shows that the better quality tea has a higher content of total essential oils and higher boiling point of aroma components (*50*). Therefore, these parameters can be used as a quality standard for tieguanyin tea from Anxi county. In this experiment, the total essential oil content of samples treated with laccase and α-galactosidase was significantly higher than in sample CK. The content of the characteristic aroma components with high boiling point, such as nerolidol, α-farnesene, phenylethyl alcohol, benzeneacetaldehyde, (Z)-3-hexenyl hexanoate, and methyl palmitate showed the same trend. This indicates that the appropriate exogenous enzyme treatment can improve the aroma of summer tieguanyin tea from Anxi county, which was confirmed by the results of sensory evaluation.

Conclusions

In conclusion, laccase and α-galactosidase significantly improved the sensory quality of tieguanyin tea from Anxi county, China, harvested in summer, which has been confirmed by sensory evaluation by the tea tasting experts and the results of main flavour substance analysis. Laccase had positive effect on the taste and α-galactosidase on the aroma. Spraying the tea leaves with the solution of the two enzymes could improve the tea quality, but the aroma of the tea remained similar to that treated with α-galactosidase alone. Treatment of tea with laccase and α-galactosidase has been rarely reported. Our study demonstrated that this treatment could improve the sensory quality of summer tieguanyin tea from Anxi county effectively. The results also show that the exogenous enzymatic treatment method can be potentially applied in other tea production processes, widening the application range of laccase and α-galactosidase.

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